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**Fate tracing reveals the endothelial origin of hematopoietic stem cells.**

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**Public Summary:**

**Scientific Abstract:**

Hematopoietic stem cells (HSCs) originate within the aortic-gonado-mesonephros (AGM) region of the midgestation embryo, but the cell type responsible for their emergence is unknown since critical hematopoietic factors are expressed in both the AGM endothelium and its underlying mesenchyme. Here we employ a temporally restricted genetic tracing strategy to selectively label the endothelium, and separately its underlying mesenchyme, during AGM development. Lineage tracing endothelium, via an inducible VE-cadherin Cre line, reveals that the endothelium is capable of HSC emergence. The endothelial progeny migrate to the fetal liver, and later to the bone marrow, and are capable of expansion, self-renewal, and multilineage hematopoietic differentiation. HSC capacity is exclusively endothelial, as ex vivo analyses demonstrate lack of VE-cadherin Cre induction in circulating and fetal liver hematopoietic populations. Moreover, AGM mesenchyme, as selectively traced via a myocardin Cre line, is incapable of hematopoiesis. Our genetic tracing strategy therefore reveals an endothelial origin of HSCs.

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